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Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Avenue N.W.
Washington, D.C. 20460

November 27, 2006

Dear Administrator:

On behalf of the Flavor and Fragrance High Production Volume Consortia, I wish to thank the Environmental Protection Agency (EPA) for their comments on the test plan and robust summaries on "Alkyl-substituted Cyclohexyl Derivatives". The Cyclohexyl Derivatives Consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for chemical substances under the Chemical Right-to-Know Program. Since 1999, the companies that are current members of the Consortium have supported the collection and review of available test data, development of test plans and robust summaries, and conducted additional testing for "Alkyl-substituted Cyclohexyl Derivatives".

Based on our initial recommendations for testing and the peer-reviewed comments of the EPA, the Cyclohexyl Derivatives Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC) is pleased to submit the following revised test plan and robust summaries for "Alkyl-substituted Cyclohexyl Derivatives". The revised test plan and robust summaries contain additional data on existing studies and the results of additional studies on toxicity, physiochemical properties and environmental fate that are related to the questions and comments made by the EPA in its letter dated 12/31/2003. This letter contains responses to the specific comments made by the EPA. These responses taken together with the inclusion of new study data and other information constitute the key changes to the original test plan and robust summaries.

With the exception of the developmental study data to be added upon completion of the final report and based other additional data, the Cycohexyl Derivatives Consortium concludes that the current test plan and robust summaries for this category is now complete. The experimental and model data for physiochemical properties, environmental fate, ecotoxicity, and human health endpoints are consistent and provide a comprehensive basis upon which to evaluate the hazard potential of both 4-t-butylcyclohexanol and 4-t-butylcyclohexyl acetate. A summary of the key hazard data has been included in this letter and also in the revised robust summaries for the cyclohexyl derivatives chemical category.

With the exception of updating the results of the developmental study (2/07), we consider that the test plan and robust summaries for this category are final and have no plans to provide additional data. The EPA comprehensive comments provided the necessary guidance to complete the test plan for this category. The collaboration between the Cyclohexyl Derivatives Consortium and the Environmental Protection Agency in the Chemical “Right to Know” Program has produced a hazard database that will be useful to the public for decades to come. Thank you for the opportunity to participate in such a program.

If you have any questions or comments concerning the contents of this letter, please feel free to contact me at any time (202-331-2325) or tadams@therobertsgroup.net.

Best regards,

Timothy B. Adams, Ph.D.

Technical Contact Person for FFHPVC

Summary of Key Hazard Data for Alkyl-substituted Cyclohexyl Derivatives

Endpoint	Substance/Surrogate[1]	Value/Range[2]	Reference
Physical Properties			
Vapor Pressure	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	<0.01 kPa (25 °C) 0.0067 kPa (20 °C)	Degussa AG, 2003a Huels AG, 1985
Partition Coefficient	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	3.23 (OECD 117) 4.8 (OECD 117)	Degussa AG, 1981 Givaudan-Roure,1996
Water Solubility	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	<100 mg/l at 20 °C ca. 90 mg/l at 20 °C	Degussa AG, 2003a Degussa AG, 2003b
Environmental Fate			
Biodegradation	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	19d/90%/(EG-Guideline 84/449/EWG C.3) 28d/75%/(EC-Guideline 92/69/E, CO2 Evolution test) 28d/54% (OECD 301F)	Degussa AG, 1983 Degussa AG,1997b Rudio, 1996a
Ecotoxicity			
Fish	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	48-hr/LC50=17 mg/L 48-hr/LC50=14 mg/L 96-hr/LC50=8.6	Degussa AG, 1987 Degussa AG, 1985a Degussa AG, 1997c
Aquatic Invertebrates	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	48-hr LC50 = 46 mg/L 48-hr LC50 = 23.4 mg/L	Degussa AG, 1994b Degussa AG, 1997a
Aquatic Plants	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	72-hr EC50 = 29mg/L 72-hr EC50 = 19 mg/L	Degussa AG, 1994a Degussa AG, 1992
Human Health			
Repeat Dose (route)	4-*t*-Butylcyclohexanol	28-d LOAEL: 150 mg/kg bw/d and NOAEL=50 mg/kg bw/d (OECD No. 407 Guideline Study)	Degussa AG, 1999

[1] Surrogate is a structurally related substance include a metabolic product or precursor of the named substance

[2] Experimental value or values for a substance or group of substances in the chemical category

Reproductive	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	NOAEL =50 mg/kg bw/d (no effects to reproductive organs and tissues) Maternal NOAEL=460 mg/kg bw/d	Degussa AG, 1999 Lewis, 2006
Developmental(route)	4-*t*-Butylcyclohexyl acetate	Maternal NOAEL=460 mg/kg bw/d Developmental NOAEL=460 mg/kg bw/d (preliminary data)	Lewis, 2006
in vitro Genotoxicity[3]	4-*t*-Butylcyclohexanol 4-*t*-Butylcyclohexyl acetate	-(AMS) - (ABS) - (AMS)	Degussa AG, 1988a Degussa AG, 1997 Degussa AG, 1989

[3] (-), no significant evidence; (+/-), equivocal evidence; (+), positive evidence of genotoxicity

EPA Comments on Chemical RTK HPV Challenge Submission:
Alkyl-substituted Cyclohexanol Derivatives Category

Summary of EPA Comments

The sponsors, Cyclohexyl Derivatives Consortium and the Flavor and Fragrance High Production Volume Cyclohexyl Derivatives Consortium, submitted a test plan and robust summaries to EPA for the alkyl-substituted cyclohexanol derivatives category dated August 21, 2003. EPA posted the submission on the ChemRTK HPV Challenge Website on August 28, 2003. The category consists of two sponsored compounds: 4-tert-butylcyclohexanol (CAS No. 98-52-2) and 4-tert-butylcyclohexyl acetate (CAS No. 32210-23-4). The submission also includes data on analogs.

EPA has reviewed this submission and has reached the following conclusions:

1. Category Justification. EPA believes that 4-tert-butylcyclohexanol and 4-tert-butylcyclohexyl acetate may be reasonably grouped into a single category based on structural similarity; however, some health effects data on various analogs do not fully support the category. Most of the data are developed either on 2-isopropyl-5-methylcyclohexanol or a mixture of seven analogs. Based on the significant difference in toxicity and potency of 4-tert-butylcyclohexanol, 2-isopropyl-5-methylcyclohexanol does not appear to be an adequate analog for 4-tert-butylcyclohexanol.

Response: The data on the isomer 2-isopropyl-5-methylcyclohexanol was intended to show that the *alpha*-2 micro-globulin phenomenon is consistent among alkyl-substituted cyclohexanol derivatives. Based on EPA response, FFHPVC has supported additional studies on the human health effects of 4-t-butylcyclohexyl acetate. Also additional data has been provided for existing studies on 4-t-butylcyclohexanol (see test plan and robust summary).

2. Physicochemical Properties. The submitter needs to provide measured values for boiling point, vapor pressure and water solubility for 4-tert-butylcyclohexanol and for 4-tert-butylcyclohexyl acetate.

Response: Additional experimental data for boiling point, vapor pressure, and water solubility of 4-tert-butylcyclohexanol and for 4-tert-butylcyclohexyl acetate have been included in the robust summaries and test plan.

3 Environmental Fate. The data for photodegradation, stability in water, and biodegradation are adequate for the purposes of the HPV Challenge Program. The submitter may need to run the fugacity model again if measured physicochemical data are developed.

Response: The EPIWIN Level III model calculation was performed using the additional experimental data for boiling point, vapor pressure and water solubility. These data can be found in the revised robust summaries.

4. Health Effects. The submitted data for 4-tert-butylcyclohexanol are adequate for acute, repeated-dose, and genetic toxicity endpoints for the purposes of the HPV Challenge Program. The submitted data for reproductive and developmental toxicity on the analog 2-isopropyl-5-methylcyclohexanol and the seven analog mixture do not adequately address these endpoints. A combined reproductive/developmental toxicity screening test is needed. The submitter needs to address deficiencies in the robust summaries.

Response: A developmental study was performed using 4-tert-butylcyclohexyl acetate. A modified OECD 421 guideline study (including ICH Harmonised Tripartite Guideline stages C and D) was used to study the reproductive/developmental toxicity of 4-*tert*-butylcyclohexyl acetate in Crl:CD(SD) pregnant female rats (Lewis, 2006). When these results are combined with the results of the 28-day repeat dose study in which no effects were seen in reproductive organs and tissues, it can be concluded that sufficient data exist to evaluate the hazard

potential of the two members of this category.

5. Ecological Effects. The submitted data are adequate for the daphnia and green algae toxicity endpoints for the purposes of the HPV Challenge Program. EPA reserves judgment on the adequacy of the fish toxicity data pending submission of critical missing data elements in the robust summary. If the information is not available, the submitter needs to perform an acute fish toxicity test.

Response: Additional data has been provided.

EPA Comments on the Alkyl-substituted Cyclohexanol Derivatives Challenge Submission

Category Definition

The submitter proposed a category consisting of 4-tert-butylcyclohexanol (CAS No. 98-52-2) and its ester, 4-tert-butylcyclohexyl acetate (CAS No. 32210-23-4). Both substances exist in *cis* and *trans* forms. The alcohol serves as a synthetic precursor of the acetate and variation in the ratio of the *cis* and *trans* isomers does not significantly alter the physical properties. The test plan also includes supporting data on the following seven alkyl-substituted cyclohexanol and cyclohexanone compounds:

(1) 2-isopropyl-5-methylcyclohexanol (CAS No. 1490-04-6);
(2) 2-tert-butylcyclohexanone (CAS No. 1728-46-7);
(3) 3-tert-butylcyclohexanone;
(4) 4-tert-butylcyclohexanone (CAS No. 98-53-3);
(5) 2-methylcyclohexanone (CAS No. 583-60-8);
(6) 3-methylcyclohexanone (CAS No. 591-24-2); and
(7) 4-methylcyclohexanone (CAS No. 589-92-4).

The submitter should have included CAS registry numbers for the supporting compounds in the test plan; EPA located CAS registry numbers for 6 of the 7 compounds.

Category Justification

The submitter supports grouping the category members based on a common use (soap perfumes) and common metabolic pathways in mammalian species. The submitter provided experimental data or estimated values for all physicochemical, environmental fate, and ecotoxicity endpoints but does not use these data to support the category. To support the common metabolism, the submitter reported that the ester undergoes rapid hydrolysis to the alcohol in *in vitro* and *in vivo* studies. In addition, the submitter provides further evidence to show that after administration of either the ester or alcohol in animal models, elimination of these two chemicals occurs through common pathways and intermediates, including the formation of glucuronic acid conjugates of cyclohexanol. The sponsor also described study data to demonstrate common metabolic and elimination pathways for related compounds such as *4-tert*-butylcyclohexanone, isomers of tert-butylcyclohexanol, and 2-isopropyl-5-methylcyclohexanol, which are used as analogs for compounds in the category. In the case of 4-tert-butylcyclohexanone, the submitter states that the ketone and 4-tert-butylcyclohexanol are interconvertible *in vivo.* Therefore, based on common chemical structures (an alkylated cyclohexanol) and metabolic pathways, the submitter expects to find similar toxicological properties for the sponsored and analog compounds.

Analog Justification

EPA believes that 4-tert-butylcyclohexanol and 4-tert-butylcyclohexyl acetate may be reasonably grouped into a single category based on structural similarity; however, some health effects data on various analogs do not fully support the category based on the following:

1. The isomer, 2-isopropyl-5-methylcyclohexanol shows different toxicity than 4-tert-butylcyclohexanol. The 28-day repeated-dose toxicity data for 4-tert-butylcyclohexanol show significant toxic effects in rats different than those for the analog, 2-isopropyl-5-methylcyclohexanol. These effects include severe neurotoxic signs such as convulsions, ataxia, fasciculation, aggressiveness, hunched posture, hypoactivity, etc. In addition, relative weights of epididymis were increased at all dose levels. These effects were not seen in the other 28-day repeated-dose toxicity study conducted on the mixture of seven components or in the 90-day repeated-dose toxicity study with 2-isopropyl-5-methylcyclohexanol. There is also a significant difference in potency of these two chemicals; 2-isopropyl-5-methylcyclohexanol being less potent (NOAELs of 750 and 1125 mg/kg/day for rats and mice, respectively) than 4-tert-butylcyclohexanol (NOAEL of 50 mg/kg/day or less

based on the changes in epididymis weights). Therefore, it is questionable whether 2-isopropyl-5-methylcyclohexanol is an appropriate analog for 4-tert-butylcyclohexanol and to extrapolate less toxic analog data to the more toxic sponsored chemicals.

Response: As stated earlier, the data on the isomer 2-isopropyl-5-methylcyclohexanol was provided to substantiate the existence of *alpha*-2 micro-globulin phenomenon in the repeat dose study for 4-t-butylcyclohexanol. In that respect, these data are consistent and one may concluded that the *alpha*-2 micro-globulin phenomenon is present in the kidney of male rats in the 28-day repeat dose study of 4-t-butylcyclohexanol. Also, there appears to be confusion concerning the change in the epididymis weights in the 28-day study. This was in part, due to the manner in which the results were reported. First, the increase in epididymis weight was observed only after the recovery period. It was not observed at the conclusion of treatment. Also, only the relative organ weight increased. There was not change in the absolute epididymis weight. In addition, the change was only observed at the highest dose. Therefore, there was no significant change to any sex organ or tissue after treatment at any dose level.

2. Reproductive and developmental toxicity endpoints. Data provided for reproductive toxicity with the mixture of seven analogs was conducted using only dosed females. The two dominant lethal assays in males (acute and subacute doses) with 2-isopropyl-5-methylcyclohexanol may not be appropriate for reasons outlined above. In all four developmental toxicity assays with 2-isopropyl-5-methylcyclohexanol, no maternal toxicity was evident at the highest tested doses which were significantly lower than the guideline recommended dose of 1000 mg/kg/day.

Overall, 2-isopropyl-5-methylcyclohexanol does not appear to be an adequate analog for 4-tert-butylcyclohexanol. In addition, most of the data are developed on either a mixture of seven analogs or on 2-isopropyl-5-methylcyclohexanol and do not support the SIDS endpoints for the sponsored chemicals. EPA recommends that the submitter conduct a combined reproductive/developmental toxicity screening test (OECD TG 421) on 4-tert-butylcyclohexanol to address these endpoints.

Response: The Cyclohexyl Derivative Consortium has taken the recommendation of the EPA and sponsored a modified OECD 421 guideline study (including ICH Harmonised Tripartite Guideline stages C and D) to study the reproductive/developmental toxicity of 4-*tert*-butylcyclohexyl acetate in Crl:CD(SD) pregnant female rats (Lewis, 2006).

<u>Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient, and water solubility).</u>

Boiling point. It is unclear whether some of the submitted data are measured or calculated. For the purposes of the HPV Challenge Program, boiling point values under 300 °C need to be measured unless precluded by experimental obstacles.

Vapor pressure for 4-tert-butylcyclohexanol and 4-tert-butylcyclohexyl acetate. It is unclear whether the vapor pressure values of less than 0.1 hPa for 4-tert butylcyclohexanol and 0.01 hPa for 4-tert-butylcyclohexyl acetate from an unpublished report are measured or calculated. The HPV Challenge Program specifies testing for substances with calculated vapor pressures above a cut-off value of $1x10^{-5}$ Pa ($7.5x10^{-8}$ mm Hg) and if the submitted data are calculated, measured data following OECD TG 104 are needed. In addition, for 4-tert-butylcyclohexanol, there is a discrepancy between the value reported in the test plan (less than 0.1kPa at 0.75 mm Hg) and that provided in the robust summary (0.1 hPa).

Water solubility for *4-tert-butylcyclohexanol* and *4-tert-butylcyclohexyl acetate.* It is unclear whether values of less than 100 mg/L at 20 °C for 4-tert-butylcyclohexanol and ca. 90 mg/L at 20 °C for *4-tert-butylcyclohexyl acetate* from an unpublished report are measured or calculated. The HPV Challenge Program specifies testing for substances with calculated water solubilities greater than 1 ug/L. If these values are calculated, the submitter needs to provide measured water solubility data for both of these chemicals following OECD TG 105.

Response: Experimental vapor pressure, boiling point, and water solubility data have been provided for both for 4-tert-butylcyclohexanol and 4-tert-butylcyclohexyl acetate (see robust summaries and test plans).

Environmental Fate (photodeqradation, stability in water, biodeqradation, fuqacity).

The data provided by the submitter for photodegradation, stability in water, and biodegradation are adequate for the purposes of the HPV Challenge Program.

Fugacity. The submitter may need to run the fugacity model again if measured physicochemical data are developed as discussed above under Physicochemical Properties.

Response: Based on the input of the additional experimental data, fugacity modeling was performed for both members of this category.

Health Effects (acute toxicity, repeated-dose toxicity, qenetic toxicity, and reproductive/developmental toxicity).

The submitted data for 4-tert-butylcyclohexanol are adequate for acute, repeated-dose, and genetic toxicity endpoints for the purposes of the HPV Challenge Program.

Reproductive/Developmental Toxicity. The submitted data are for the analog 2-isopropyl-5-methylcyclohexanol or for the analog mixture and EPA does not believe that these are appropriate analogs for the sponsored substances (see analog justification). Consequently, EPA recommends that the submitter conduct a combined reproductive/developmental toxicity screening test (OECD TG 421) on 4-tert-butylcyclohexanol to address these endpoints.

Response: The EPA recommendation has been taken. An OECD No. 421 has been performed.

Ecological Effects (fish, invertebrates, and algae).

The submitted data are adequate for the daphnia and algae toxicity endpoints for the purposes of the HPV Challenge Program. EPA reserves judgement on the adequacy of the 4-tert-butylcyclohexyl acetate *Cyprinus carpio* study pending submission of critical missing data elements in the robust summary. If the specified information is not available, the submitter needs to perform a fish toxicity test according to the OECD guideline to adequately addresses this endpoint. (The two studies of 4-tert-butylcyclohexanol, submitted on the Golden orfe are inadequate to address the fish toxicity endpoint.)

Response: Additional data has been added to the 4-t-butylcyclohexyl acetate 96-hr acute toxicity study. Certainly the order of acute toxicity observed in this study is consistent with the lower order of toxicity observed in two static 48-hour toxicity studies for the corresponding alcohol. Based on these data the 96-hour LC50 for the category can be estimated to be 5-10 mg/l.

Specific Comments on the Robust Summaries

Health Effects

None of the summaries listed the purity of the test material and none of the summaries for studies on analogs provided the CAS registry number of the analog.

Acute Toxicity. Robust summaries for acute oral toxicity studies (one for 4-tert-butylcyclohexanol and three for 4-tert-butylcyclohexyl acetate) in rats were missing the following information: doses administered, length of the observation period, gavage vehicle (if used), sex and strain of rat, group size, results for mortality by sex and dose, and method for calculating the LD50. The summary for the study by Opdyke (1976) mis-stated the LD50 in the Value field as *less than 5 but greater than 500 mg/kg;* this should probably read *less than 5 g/kg but greater than 500 mg/kg.*

Response: These data were added where available. The LD50 study has been corrected to reflect the error in the robust summary.

Genetic Toxicity. Robust summaries were missing the following information: all of the tested concentrations (a range was given), and the criteria for a positive result. The Remarks fields included a statement on the number of metaphases analyzed, which is irrelevant to prokaryotic systems and should be deleted.

Response: These data were deleted.

Ecological Effects

Fish. Information missing from the robust summary of the studies of 4-tert-butylcyclohexyl acetate in *Cyprinus carpio* includes test substance purity, control use/response, water hardness, test temperature, organism specifications, mortality at each concentration, and statistical methods used.

Response: The additional data were included in the robust summary when available.

Invertebrates. The submitter needs to identify a key study for this endpoint and provide the following missing information in the robust summaries: the number of daphnids, concentrations tested, control use/response, effects (percentage immobilization) at each concentration, temperature, and statistical methods used. In addition, in the summaries of the studies on 4-tert-butylcyclohexanol and *4-tert*-butylcyclohexyl acetate, it was not clear whether the pH and the dissolved oxygen ranges were for the entire test duration, and whether the dissolved oxygen values represented at least 60% of air saturation at test temperature.

Algae. Information missing from one or more of the summaries includes water quality characteristics (pH and test temperature), light intensity and quality, details about cell density/inhibition of cell growth at all

concentrations tested, control use/response, statistical methods used, and whether or not the EC50 values were based on nominal or measured concentrations. The submitter needs to provide this missing information.

Response: The additional data were included in the robust summary when available.